A SERIES OF OLIGOSACCHARIDES, OCCURRING IN THE HONEYDEW OF INSECTS, BASED ON TURANOSE

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Synopsis

A series of oligosaccharides, occurring in the honeydew of various insects, is described. Each oligosaccharide yields, on hydrolysis, the disaccharide turanose and a second fragment consisting of from one to five glucopyranose units. These oligosaccharides have been found to occur in the honeydew of sap-sucking insects of several different Families.

The parent sugar, turanose, and two members of the series are already known. This paper names and describes two new members of the series, cryptose, a pentasaccharide, and lasiose, a hexasaccharide, and foreshadows a still higher member.

The honeydew excreted by several sap-sucking insects is a syrup containing a number of sugars, the composition of which differs very markedly from that of the sap ingested by the insect. The sugars in the sap of several eucalypts have been reported (Basden, 1965) as consisting of approximately 10% raffinose, 80% sucrose and 10% of a mixture of glucose and fructose. The honeydew of the insects feeding on the sap of these eucalypts contains about 10% sucrose, 10% raffinose and the remainder consists of sugars of high molecular weight.

The present investigation commenced with the examination of the honeydew of *Eriococcus coriaceus* Mask. (Basden, 1967). It was shown that the honeydew contained several sugars which were not present in the sap, nor indeed had been previously reported as occurring in nature. These sugars have been found subsequently to occur in the honeydew of several insects and of different Families as shown in Table 1.

TABLE 1
Insects, the Honeydew of which Contains the Sugars Described in this Paper

Insect	Family
Siphonophora rosae Linn.	*Aphididae*
A mealy bug	*Coccidae*
Cryptes baccatus Mask.	*Coccidae*
Eriococcus coriaceus Mask.	*Coccidae*
Eucalyptolyma maidenii Frogg.	*Psyllidae*
Cardiaspina densitexta Taylor	*Psyllidae*
Lasiopsylla striatus Frogg.	*Psyllidae*

Two of the sugars, turanose and laminaribiose, had been prepared in the laboratory but had not been isolated as such from a natural source. Another sugar, eriose, had not been previously reported at all. Since the above paper was published two further oligosaccharides have been identified. One, for which the name cryptose is proposed, is a pentasaccharide and the other, lasiose, is a hexasaccharide. There is evidence of the occurrence of a still higher oligosaccharide, but it is in such small amount that its separation and examination at present are impracticable.

These two oligosaccharides, cryptose and lasiose, as well as eriose and melezitose, which have been previously described, are all easily hydrolysed and all yield the disaccharide turanose as one of the products, the other fragment of the molecule being, as shown in Table 2, either glucose or one of the 3-D-glucopyranosides, each being one glucopyranosyl unit larger than its predecessor in the group. The identity of the turanose, glucose, laminaribiose and laminaritriose have been proved by comparison with authentic specimens of the respective sugars. As no authentic laminaritetraose has been available, the identity of this sugar has been assumed on the following grounds :

(1) From analogy with laminaribiose and laminaritriose in the series.
(2) It gives the characteristic colour of the laminarin sugars on treatment with aniline-diphenylamine phosphate reagent.
(3) It occupies the typical position of a tetrasaccharide on the chromatogram.
(4) Its molecular weight is that of a tetrasaccharide.

TABLE 2
The Sugars of the Turanose Series

Sugar	Structure	Structure Determined by	Products of Hydrolysis
Turanose ..	O–D–Gp(1→3)Fru.f	Hudson 1944	
Melezitose ..	O–D–Gp(1→3)Fru.f(2→1) O–D–Gp	Hehre 1953	Turanose, glucose
Eriose ..	O–D–Gp(1→3)Fru.f(2→1) O–D–Gp(3→1)O–D–Gp	Basden 1967	Turanose, laminaribiose
Cryptose ..	O–D–Gp(1→3)Fru.f(2→1) O–D–Gp(3→1)O–D–Gp(3→1) O–D–Gp		Turanose, laminaritriose
Lasiose ..	O–D–Gp(1→3)Fru.f(2→1) O–D–Gp(3→1)O–D–Gp(3→1) O–D–Gp(3→1)O–D–Gp		Turanose, laminaritetraose

The symbols Gp and Fru.f represent glucopyranose and fructofuranose respectively and are the symbols prescribed in The Handbook for Chemical Society Authors 1960.

When more of the material has been accumulated, the composition will be verified.

The classical method of separation of sugars from a mixture by percolation through a column of Celite and charcoal, using increasing strengths of aqueous ethanol as eluent has been found impracticable due to the small differences in mobility of several of the components of the honeydew.

The sugars were satisfactorily separated by paper chromatography, sometimes repeated three times. Experimental details are given later in this paper.

Sufficient of only one of the sugars, cryptose, has been obtained to enable its physical properties to be determined. Specimens of eriose and lasiose are being collected as opportunity occurs and will be examined when sufficient of both has been collected.

Cryptose. $C_{30}H_{52}O_{26}$

This sugar, named from *Cryptes baccatus* Mask., in which it occurs, crystallizes from a concentrated aqueous solution as fine needles generally associated in rosettes. These crystals are soluble in water, pyridine and slightly in methanol. They are insoluble in ethanol. They have a faintly sweet taste. Cryptose does not reduce Fehling's solution. Its melting point is 160° C. and its $[\alpha]_D^{25}$ +110·7 (C=4).

Cryptose is separated from the other sugars of *Eriococcus* honeydew by paper chromatography. A streak of honeydew solution is made on Whatman's no. 3 chromatograph paper and this is treated as a descending chromatogram at room temperature. The solvent found most efficient was butanol : acetone : water, 3 : 4 : 1. It was run for 48 hours, the paper dried, and the area occupied by cryptose was located, removed and extracted with water in a Soxhlet extractor. The solution was decolourized with charcoal and then evaporated to a syrup. It is important to use charcoal which has had any residual acidity neutralized as cryptose readily hydrolyses in an acid medium. On standing several days at room temperature, the sugar crystallized from the syrup as rosettes of fine colourless needles.

Cryptose has been proved to be a pentasaccharide by the determination of its molecular weight. Using a Metrolab Model 302 Vapour pressure Osmometer, the molecular weight was found to be 820 (calculated 828). On a paper chromatogram using butanol : acetone : water, 3 : 4 : 1 as solvent at room temperature cryptose was found to have a motility $R_G = 0 \cdot 19$ and on development with aniline-diphenylamine phosphate it yielded a mauve stain classified as Victorian mauve B.C.C. 297 of the British Colour Council Dictionary of Colours. This colour must be observed as soon as it is formed as it quickly darkens on exposure.

Lasiose. $C_{36}H_{62}O_{31}$

This sugar, named from *Lasiopsylla striatus* Frogg. in the honeydew of which it occurs, was separated from the crude *Eriococcus* honeydew in a manner similar to that employed for cryptose. From the intensity of colour and the size of the stain on a paper chromatogram it is estimated that lasiose comprises less than 10% of the total sugar content of the honeydew. Lasiose is readily hydrolysed by dilute acid, yielding turanose and another sugar which has been assumed to be laminaritetraose. Insufficient lasiose has been obtained to permit an examination of its physical properties other than to observe that it crystallizes in (apparently) monoclinic plates. These are soluble in water, in pyridine, and slightly soluble in methanol. They are insoluble in ethanol. On a paper chromatogram at room temperature and with butanol : acetone : water, 3 : 4 : 1 solvent it has a R_G $0 \cdot 01$ and yields a Victorian mauve stain with the aniline-diphenylamine reagent. It is of interest to note that the honeydew of every insect examined contains, in addition to the series of sugars named above, about 10% raffinose. This is approximately the proportion present in the original sap and seems to indicate that the raffinose passes through the alimentary system of the insect unchanged and that the oligosaccharides of the turanose series are derived from sucrose. No indication has yet been obtained as to the manner in which sucrose is broken down and the fragments recombined to form the complex oligosaccharides.

The hydrolysis of all the sugars in this turanose series was effected by heating a fragment of the sugar or a drop of its solution with $0 \cdot 05$ M sulphuric acid at 100° C. for five minutes. The solution was neutralized with barium carbonate, separated from the precipitate on a centrifuge, and the supernatant solution evaporated and chromatographed on Whatman's no. 1 paper using authentic samples of turanose, laminaribiose and laminaritriose as controls. The location of the various sugars on the chromatogram was determined by dipping the dried chromatogram in aniline-diphenylamine reagent as prescribed by Bailey and Bourne (1960) and comparing the colours and positions of the standards with those of the sugars under test.

References

Bailey, R. W., and Bourne, E. J., 1960.—Colour reactions given by sugars and diphenylamine-aniline sprays on paper chromatograms. *J. Chrom.*, 4 : 206.

Basden, R., 1965.—The occurrence and composition of manna in *Eucalyptus* and *Angophora*. Proc. Linn. Soc. N.S.W., 90 : 152.

————, 1967.—The occurrence and composition of the sugars in the honeydew of *Eriococcus coriaceus* Mask. Proc. Linn. Soc. N.S.W., 92 : 222.

Hudson, C. S., 1944.—Turanose. *J. Org. Chem.*, 9 : 117.

Hehre, E. J., 1953.—Melezitose. *Adv. in Carbohydrate Chemistry*, 8 : 277. Academic Press Inc., N.Y.